Remarks / Arguments

Claims 82, 84-96, and 98-120 are pending in this application. Claims 82, 85-88, 90, 93, 96, 98, 100, and 102, have been withdrawn from consideration.

Applicants respectfully request reconsideration of claims 84, 89, 91-92, 94-95, 99, 101 and 103-120 based on the following remarks.

I. Maintained Grounds of Rejections

a) Rejections Under 35 U.S.C. §112, First Paragraph

Claim 109 stands rejected under 35 U.S.C. § 112, first paragraph. The Examiner opines that the specification lacks written description to show the inventors were in possession of the claimed invention at the time the application was filed.

Applicants respectfully traverse this ground of rejection.

Applicants respectfully direct the Examiner's attention to page 43, line 17 through page 46, line 19 of the specification, part of Example 1 describing the generation of dendritic cells from murine blood. At page 45, line 2, the specification states that the cells are ready to be subcultured at "about day 10". They are ready to be subcultured again "at about 20 days" (see page 45, lines 25-26). The specification further states that "colonies of growing cells, as confirmed by 3H-Tdr labeling and audioradiography [below], could be generated in subcultures for 1-2 months" page 45, lines 32-34).

Applicants respectfully posit that if the specification teaches that cell aggregates are subcultured about once every 10 days for two months, then the ordinarily skilled artisan would realize that they are subcultured from about one to five times.

Further, at page 57, lines 20-22 (part of Example 2 describing the generation of dendritic cells from murine bone marrow cultures), the specification states, "To enrich for growing dendritic cells, we utilized a procedure similar to that described for the mouse blood cell cultures

of Example 1." In other words, the specification teaches, again, that the cell aggregates are subcultured from about one to five times.

Accordingly, Applicants posit that they clearly possessed, and described, the subculture of cell aggregates one to five times in the specification. Based on these remarks, Applicants respectfully request that this ground for rejection be reconsidered and withdrawn.

Claims 84, 89, 91-92, 94-95, 99, 101, and 103-120 stand rejected under 35 U.S.C. §112, first paragraph, as lacking written description because the disclosure allegedly does not reasonably convey to the ordinarily skilled artisan that the inventors were in possession of the claimed invention at the time the application was filed. Specifically, the Office Action has asserted that there is no support for "A composition comprising an enriched and expanded population of antigen-activated dendritic cells." (Office Action, p. 3).

Claims 84, 89, 91-92, 94-95, 99, 101, and 103-120 also stand rejected under 35 U.S.C. §112, first paragraph, because there is allegedly insufficient written description to show that the Applicants possessed "a modified antigen" or "antigen modification" (Office Action, p. 3).

Applicants respectfully traverse both of these grounds of rejection.

Applicants have addressed these two grounds of rejection together because they relate to the same issue, namely, what is, in fact, Applicants' described and claimed invention.

The invention described and claimed by the Application relates to the utilization of dendritic cells as antigen presenting cells. The Application teaches the development of the antigen presenting cells by first describing the production of an enriched and expanded population of dendritic cell precursors. Examples 1 and 2 in the specification (spanning page 43, line 17 through page 65, line 4) describe the production of this enriched and expanded population of dendritic cell precursors murine blood and bone marrow. Subsequent examples (*e.g.*, Example 3, spanning page 65, line 5 through page 73, line 18) describe pulsing (*i.e.*, culturing) the dendritic cell precursors *in vitro* with BCG mycobacteria. In Example 3, at page 69, line 16

through page 73, line 18, the ability of these antigen-activated dendritic cells (*i.e.*, the dendritic cell precursors pulsed with BCG mycobacteria) to serve as antigen presenting cells to T cells was shown *in vitro* as well as *in vivo* (see, particularly page 71, line 21 through page 73, line 18).

The fact that T cells could specifically recognize and respond to the dendritic cell antigen-activated dendritic cells makes evident the fact that the BCG mycobacteria was modified by the dendritic cells, since it is well known that T cells can only recognize peptide antigens presented on the surface of a cell in the context of an MHC class I or II molecule (see, *e.g.*, Fundamental Immunology, 3rd Ed. (William E. Paul, ed.), Raven Press, New York 1993, page 383 (cover pages and page 383 attached herewith as Appendix A)). As is also well known, MHC class I and class II molecules bind to peptides of about 8-11 amino acids length, or about 10-30 amino acids in length, respectively (see, *e.g.*, Fundamental Immunology, 3rd Ed. (William E. Paul, ed.), Raven Press, New York 1993, pages 642-643 (cover pages and pages 642-643 attached herewith as Appendix B)).

Applicants further aver that one of ordinarily skill in the art, at the time of the invention, would realize that the live BCG mycobacteria used by the Applicants (see, *e.g.*, page 66, lines 8-11) are comprised of proteins larger than 30 amino acids in length. Indeed, the specification specifically contemplates that most immunogens must be first modified by being processed within the dendritic cell precursor in order to be presented on the cell surface of the now antigen-activated dendritic cell (see, *e.g.*, specification at page 36, lines 13-15: "Processing of antigen by dendritic cells or dendritic cell precursors includes the fragmentation of an antigen into antigen fragments which are then presented.")

Further, Applicants posit that the specification's pulsing of dendritic cell precursors with BCG mycobacteria to result in antigen-activated dendritic cells is merely one example of an infinite number of different immunogens that can be used to generate antigen-activated dendritic cells. Applicants further note that just because one organism, namely the BCG mycobacterium, was used to generate the antigen-activated dendritic cells described in Example 3 of the specification, this certainly does not mean that all of the antigen-activated dendritic cells produced are presenting the same BCG mycobacteria antigen. On the contrary, the ordinarily skilled artisan, upon reading in the specification at page 71, line 21 through page 72, line 11, that

a sizeable T cell response in an *in vitro* mixed leukocyte reaction was elicited by a mere 100 antigen-activated dendritic cells, would be led to conclude that many T cells bearing T cell receptors of different specificity were reacting to these antigen-activated dendritic cells (in other words, the ordinarily skilled artisan would realize that the vast number of T cells responding were not a clonal population).

Thus, Applicants clearly contemplated that invention included all antigen-activated dendritic cells, and not just those antigen-activated dendritic cells expressing a modified antigen from BCG mycobacteria. The generation of antigen-activated dendritic cells expressing modified antigens is described in detail in the specification at page 34, line 33 through page 42, line 8.

Accordingly, Applicants respectfully aver that the specification fully supports "A composition comprising an enriched and expanded population of antigen-activated dendritic cells", as well as "a modified antigen" or "antigen modification". Based on these remarks, Applicants respectfully request reconsideration and withdrawal of these 35 U.S.C. §112, first paragraph, written description rejections.

b) Nonstatutory Double Patenting Rejection

Claims 84, 89, 91-92, 94-95, 99, 101, and 103-120 stand provisionally rejected for obvious-type double patenting over claims 45 and 46 of copending Application No. 10/287,813.

The Examiner has acknowledged that Applicants have requested that this ground for rejection be held in abeyance until allowable subject matter is indicated.

II. New Grounds of Rejections

a) Rejection Under 35 U.S.C. § 103(a)

Claims 84, 89, 91-92, 94-95, 97, 99, 101, and 103 stand rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Sallusto *et al., J. Exp. Med.* **179**: 1109-1118, 1994 ("Sallusto"), in view of Aldovini *et al., Nature* **351**: 479-482, 1991 ("Aldovini"), as evidenced by Inaba *et al., J. Exp. Med.* **172**: 631-640, 1990 ("Inaba").

Applicants respectfully traverse this ground of rejection.

Applicants respectfully point out that Sallusto is not a proper prior art reference to this Application. Sallusto was published in April 1994. The present Application is a divisional application of U.S. application serial no. 08/458,230 filed June 2, 1995 (now U.S. Patent No. 5,851,756), which is a continuation of U.S. application serial no. 08/040,677 filed March 31, 1993. Applicants note that although the current filing receipt on file is incorrect and that Applicants sought to correct this error in a Request for Corrected Filing Receipt mailed Dec. 4, 1998 (copy enclosed as Appendix C). Applicants are, concurrent with this response, filing a Second Request for Corrected Filing Receipt.

Accordingly, as no new matter has been added to the Application since March 31, 1993, Applicants respectfully aver that the claims at issue were fully supported and enabled by U.S. application serial no. 08/040,677. Therefore, Sallusto is not prior art to the present Application.

Further, Applicants respectfully note for the record that the broadest claim (*i.e.*, claim 101) at issue does not require the use of a mycotuberculosis (*e.g.*, the BCG) antigen. Accordingly, although the Examiner has acknowledged that Sallusto does not teach the use of a mycotuberculosis (specifically BCG) antigen, this limitation should not be read into the present claims. Applicants respectfully aver that Sallusto is different from the claimed invention for reasons other than the failure of Sallusto to teach a mycotuberculosis antigen.

Because the primary reference, namely Sallusto, is not a proper prior art reference to this Application, this ground of rejection has been rendered moot and Applicants respectfully request its withdrawal.

b) Rejections Under 35 U.S.C. §112, First Paragraph

Claims 110, 115, 118, and 119 stand rejected under 35 U.S.C. §112, first paragraph, for lack of written description because the claims allegedly contain new matter.

Specifically, the specification and original claims allegedly do not provide support for the phrase "wherein the cell aggregates are subcultured about every 3 to 30 days" in Claim 110. This limitation is allegedly only described in the specification for a "blood derived population of dendritic cells" (Office Action, p. 6).

Applicants respectfully traverse this ground of rejection.

The specification, at page 44, line 1 through page 46, line 19, describes subculturing cell aggregates derived from blood. Further, at page 57, lines 20-22, the specification teaches that the procedure used to isolate and enrich dendritic cells from blood can also be employed to isolate an enrich dendritic cells from bone marrow cultures.

Thus, the specification fully supports the subculturing about every 3 to 30 days of cell aggregates, where the cell aggregates are derived from a tissue source comprising dendritic cell precursors, such as blood or bone marrow.

Based on these remarks, Applicants respectfully request reconsideration and withdrawal of this ground of rejection.

Further, the specification and original claims have been alleged to not provide support for the phrase "wherein said modified antigen is presented by the dendritic cells on MHC class I and MHC class II" in Claim 115. The Office Action asserts that the specification discloses this limitation only for a microbial, other, and recombinant viral antigens (Office Action, p. 6).

Applicants respectfully traverse this ground of rejection.

Applicants respectfully point out that it was well known at the time of the invention that T cells recognize antigen only in context of MHC class I and MHC class II (see Appendix A and

comments above). Thus, Applicants respectfully aver that the ordinarily skilled artisan would understand that the statement in the specification at page 41, lines 29-34:

> The novel antigens of the invention are prepared by combining substances to be modified or other antigens with the dendritic cells prepared according to the method of the invention. The dendritic cells process or modify antigens in a manner which promotes the stimulation of T-cells by the processed or modified antigens

to mean that the modified antigen could only be presented by the dendritic cell in context of MHC class I and MHC class II.

As the Federal Circuit has repeatedly stated, the disclosure does not have to provide *in haec verba* support for the claimed subject matter at issue to satisfy the written description requirement (see, *e.g.*, Purdue Pharma L.P. v. Faulding Inc., 230 F.3d 1320 (Fed. Cir. 2000). Further, it is a long-standing premise that a "patent need not teach, and preferably omits, what is well known in the art." Hybritech Inc. v. Monoclonal Antibodies, Inc., 802 F.2d 1367 (Fed. Cir. 1986).

Accordingly, based on these remarks, Applicants respectfully request reconsideration and withdrawal of this ground of rejection.

Finally, the specification and original claims have been alleged to not provide support for the phrase "wherein the dendritic cell precursors are cultured in the presence of antigen" in Claims 118-119. Rather, the specification allegedly discloses this limitation only for a "particulate matter" (Office Action, p. 3).

Applicants respectfully traverse this ground of rejection.

Applicants respectfully point out that the particulate matter described in the specification at page 66, lines 8-19 *is* the antigen (*i.e.*, the particulate matter is 10^7 live BCG mycobacteria). That is why dendritic cells precursors cultured with this "particulate matter" are able to present BCG antigen both *in vitro* and *in vivo* to T cells (see page 71, line 21 through page 73, line 18).

Applicants also direct the Examiner's attention to the teachings of the specification from page 36, line 16 through page 39, line 30, which relate to the culture of dendritic cell precursors with antigen in order to present antigen on MHC class I or class II molecules on the dendritic cell surface. The dendritic cell precursors uptake the antigen with which they are cultured, modify them (*e.g.*, by fragmenting the antigen into peptide fragments capable of binding to MHC class I or class II molecules), and present them to T cells.

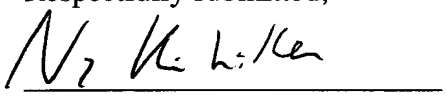
Thus, Applicants respectfully aver that the specification fully supports the phrase "wherein the dendritic cell precursors are cultured in the presence of antigen." Based on these remarks, Applicants respectfully request reconsideration and withdrawal of this ground of rejection.

Conclusion

In view of the foregoing remarks, Applicants respectfully submit that this application is in condition for allowance. If a telephone interview would advance prosecution of the application, the Examiner is invited to call the undersigned at the number listed below.

This response is being filed on Monday, Nov. 15, 2004 (Nov. 13, 2004 being a Saturday). Thus, no fees are believed to be due in connection with the filing of these papers; however if there are any other fees (or overpayments), please charge (or credit) the fees to our Deposit Account No. 08-0219.

Respectfully submitted,



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Appendix A

FUNDAMENTAL IMMUNOLOGY

THIRD EDITION

Editor

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CHAPTER 11

T Lymphocyte Antigen Receptors

Stephen M. Hedrick and Frank J. Eidelman

Antigen is recognized by T cells in the form of an oligopeptide bound to major histocompatibility complex (MHC) molecules. This simple statement depends on years of experiments that have explored the peptide specificity of T cells (1–4), the dependence of antigen recognition on MHC allelic variations (5,6), the requirements for antigen processing (7–9), and the three-dimensional structure of MHC molecules (10,11). The antigen–MHC determinant is recognized by a disulfide-bonded heterodimer encoded by genes that are specifically rearranged and expressed in T cells (12,13). This heterodimer is part of a receptor complex consisting of as many as seven different polypeptide chains associated in the cell membrane (14). The focus of this chapter is a description of the genetics, biochemistry, function, and specificity of the T cell antigen receptor (TCR).

There are two types of T cells that are defined by the form of the TCR heterodimer expressed. T cells that express the TCR $\alpha\beta$ heterodimer account for all of the known antigen-specific regulatory and effector functions that are well characterized for cellular and humoral immune responses. These T cells respond to antigen in asso-

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Appendix B

FUNDAMENTAL IMMUNOLOGY

THIRD EDITION

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ity in this assay when T cells of distinct specificity were used as effector cells (70,71). These data provided clear evidence that biologically active, and presumably intact, peptides could be recovered from class I molecules after natural intracellular processing and binding and suggested that if sufficient material could be obtained, it would be possible to chemically characterize the recovered peptides. An unexpected and still not fully understood observation was made in the course of extending these observations. When one looked for specific peptides usually associated with a particular class I molecule in cells synthesizing the known source proteins but lacking the relevant class I molecule, such peptides could not be found (72). Thus the accumulation of a detectable amount of the specific short peptides found stably associated with class I molecules required those class I molecules to be present and to capture the peptide. Otherwise, such peptides are either not generated or degraded too rapidly to reach a steady-state level of even one to two copies per cell.

The first application of the elution approach to the identification of a specific naturally processed viral antigenic peptide did not involve direct sequencing of the eluted peptide but rather the use of the characteristic HPLC elution position of a naturally processed influenza hemagglutinin determinant to identify a synthetic peptide with identical chromatographic properties. This peptide corresponded to a nonamer within the stretch of amino acids of the hemagglutinin molecule previously shown to contain the T cell determinant recognized by the indicator T cell clone (73). A refinement of this approach involved the application of microsequencing to peptides eluted from isolated H-2K^b molecules of metabolically radiolabeled cells infected with vesicular stomatitis virus, which revealed an octamer with tyrosines at positions 3 and 5 and leucine at position 8, corresponding to a core sequence present in synthetic peptides already known to be capable of sensitizing cells for recognition by vesicular stomatitis virus-specific, H-2K^b-restricted CTLs (74).

Two distinct methodologies subsequently have been used to gather sufficient masses of data to identify common structural characteristics of the peptides bound to a single allele of class I molecule. Falk et al. (75) subjected pools of peptides eluted from single class I alleles to Edman degradation. Although unique sequences were not obtained from these heterogeneous mixes, particular amino acids gave very strong signals at specific positions, and the sequencing signals did not usually extend beyond position 9. The occurrence of strong signals at a given position in the sequence indicated that the NH_2 termini were a fixed number of amino acids from these common residues in each peptide. The predominant residues could be found in comparable positions in most peptides previously identified as being presented by the relevant class I molecule. This gave rise to the concept of "motif" or "anchor" amino acids important in promoting peptide binding to a particular allele of class I, and such residues identified by sequence analysis have been found to correspond to the residues whose side chains occupy the major binding pockets in the class I-binding site. Thus there is satisfying concordance between the molecular images we have of peptide–MHC class I interactions and the functional behavior of class I molecules in cells in terms of selective capture of peptides with particular chemical structures. This correspondence extends beyond the allele-specific motifs that involve binding to the polymorphic pockets of the class I-binding groove. The lack of strong sequencing signals beyond 9 amino acids in the pools of eluted peptide fits well with the 8- and 9-residue length of the specific viral peptides found associated with class I molecules, and with the crystallographic picture of bound peptides with anchored N and C termini making hydrogen bonds to the conserved residues at either end of the binding groove.

Two distinct techniques have also been used to identify and characterize large numbers of individual peptides associated with particular allelic forms of class I. In one approach, HPLC-purified single peptide peaks have been sequenced by chemical degradation, revealing additional motifs for many HLA and H-2 class I alleles (Table 1). An even more powerful method has been de-

TABLE 1. *Anchor/motif residues involved in allele-specific binding of peptides to MHC molecules as determined by direct peptide sequencing*

MHC class	Allele	Motif	Pocket
Class I	HLA-A2	P2 = L	B
		P9 = V	F
	HLA-B27	P2 = R	B
		P9 = K, R	F
	HLA-A3	P2 = L	B
		P9 = Y, K	F
	H-2D^b	P5 = N	C
		P9 = M	F
	H-2K^b	P5 (or 6) = Y, F	C
		P8 (or 9) = L	F
	H-2K^d	P2 = Y	B
		P9 = I, L	F
	H-2L^d	P2 = P	B
		P9 = F	F
	H-2D^d	P2 = G	B
		P3 = P	B
		P5 = + charge	D
		P9 = L	F
Class II	I-A^b	xxNxxxxxPxxxx	
	I-A^s	xxxxITxxxxHxxx	
	I-E^b	xxYIYxxxxRRxxYx	

Class I motifs are given by peptide residue position ("P") and binding groove pocket. Class II motifs are given as core sequences with x representing nonconserved residue and capital letters indicating conserved motif residues. No precise length position or pocket assignment can be made for class II peptides.

veloped involving microcapillary HPLC and electrospray ionization–tandem mass spectrometry to evaluate both the complexity of bound peptides as well as their sequences. This methodology permitted measurement of a minimum of 200 different peptides copurifying with HLA-A2 and the sequencing of numerous individual peptide species (76).

These studies have also revealed the source proteins of many of the isolated peptides, based on homology or identity to sequences contained in DNA or protein databases (Table 2). Of the peptides whose source protein could be unambiguously identified by this approach, all but one are from abundant cytoplasmic or nuclear proteins, as expected from the general model in which such intracellular proteins serve as the major source of peptide for presentation by the class I pathway. Exceptions to this general finding in normal, and especially mutant, cell lines will be discussed in a later section.

Peptides Associated with Class II Molecules

The same methods used to analyze class I-associated peptides have been employed to study the peptides naturally bound to class II molecules. Immunoaffinity-purified class II molecules have been treated with acid and the eluted peptides analyzed by Edman degradation or mass spectroscopy (77–80). Such analyses have shown that each MHC allele gives a characteristic profile of eluted peptides, consistent with the role of allelic polymorphism in "determinant selection." In contrast to the situation with class I, class II bound peptides are longer and more heterogeneous in size, ranging in length from 12 to more than 24 residues. This heterogeneity is not simply the result of differing lengths for peptides derived from distinct proteins but also reflects the capture of nested peptide sets from a single protein, that is, peptides that share a core of amino acids but have different overall lengths and N and C termini. Both amino- and carboxy-terminal extensions are observed, suggesting that class II lacks a requirement for the relatively buried, conserved bonds formed at the ends of the class I binding groove that play a predominant role in stabilizing interaction with peptide. These data are consistent with the emerging crystallographic findings that class II has a more "open" structure that allows these longer peptides to lie in the binding groove without substantial kinking.

Because class II-associated peptides with common core sequences show staggered NH_2 termini, it is difficult to carry out sequencing of mixed pools of eluted peptides to identify motifs or anchor residues. Therefore distinct peptide species have usually been sequenced after HPLC or mass spectroscopic isolation, and the sequences aligned by sliding the core regions of the overlapping sequences with respect to one another. Using this method, it has been possible to identify some possible motifs for binding to particular class II alleles (Table 1). These generally involve only 2 to 3 residues from among a core region of 7 to 9 residues, consistent with studies using synthetic peptides. The motif residues are presumed to occupy polymorphic pockets of the type identified in the class I binding site.

The sources of peptides bound to class II molecules fit with the general model of class II function and the class II processing pathway, in that the predominant identifiable species are derived from proteins with ready access to the endocytic pathway (Table 2). This implies that intracellular proteins access the class II pathway inefficiently compared to the class I pathway. However, very few of the less abundant peptides associated with class II have been analyzed, and these might prove to come in part from such proteins. As for class I molecules, differences are observed in the spectrum of peptides associated with class II molecules from normal and mutant cell lines with altered antigen presentation, and these will be discussed in a later section.

TABLE 2. *Sources of peptides found naturally associated with class I and class II MHC molecules*

Class I associated	Class II associated
Ribosomal protein 60S (cytosolic)	Retroviral envelope (integral membrane protein)
Heat-shock protein HSP89α (cytosolic)	MHC class II Eα (integral membrane protein)
Heat-shock protein HSP89β (cytosolic)	Invariant chain (integral membrane protein)
Elongation factor 2 (cytosolic)	Bovine serum albumin (prevalent serum component)
Helicase (nuclear)	Apolipoprotein (prevalent serum component)
Histone H3 (nuclear)	Transferrin receptor (integral membrane protein)
Signal sequence (ER)	MHC class I heavy chain (integral membrane protein)
Leukocyte common antigen (endogenously synthesized membrane protein)	Immunoglobulin heavy chain (integral membrane protein)
Phosphoglycerate kinase (cytosolic)	Na^+/K^+ ATPase (integral membrane protein)

PEPTIDES AS INTEGRAL PARTS OF MHC CLASS I AND CLASS II MOLECULAR STRUCTURE

Both the visualization of peptides in class I MHC molecule crystals, the ability to nearly quantitatively recover peptides from MHC molecules even after the harsh treatments involved in affinity purification, and the very slow dissociation rates measured for MHC class II–synthetic peptide complexes all gave evidence of the

Appendix C